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Comparative Antioxidant and Anti-Inflammatory Activity of Ellagic Acid and *Juglans regia* L. in Collagenase-Induced Osteoarthritis in Rats

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Abstract: Osteoarthritis (OA), the most common degenerative pathology of the joints, affects mainly elderly people, and it is one of the most important factors causing disability. This study aimed to assess the effect of *Juglans regia* L. on rats with collagenase-induced knee osteoarthritis comparative with groups with the same disease treated with ellagic acid (EA), indomethacin as positive control and vehicle as negative control. After 2 and 4 weeks of treatment, blood samples were collected in order to evaluate the oxidative stress and inflammation, as well as RANKL and hydroxyproline levels. The results showed that EA improved the systemic antioxidant defense ($p < 0.05$), decreased the interleukin-6 (IL-6) secretion ($p < 0 < 0.05$) and RANKL levels ($p < 0.01$ and $p < 0.001$) at the same time enhancing hydroxyproline values, particularly after 2 weeks of treatment ($p < 0.01$). JR extract especially maintained low values of RANKL ($p < 0.05$) and hydroxyproline levels ($p < 0.05$), indicating a partial chondroprotective effect compared to EA. In conclusion, the use of EA and JR extract can improve some parameters of bone regeneration in experimental osteoarthritis, suggesting beneficial effects in articular inflammatory diseases. However, further studies are necessary to establish the optimum dose and time of treatment with both compounds in order to obtain optimal therapeutic results.

Keywords: osteoarthritis; *Juglans regia* L. extract; ellagic acid; malondialdehyde; superoxide dismutase; catalase; tumor necrosis factor-α; interleukin-6; RANKL; hydroxyproline

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Academic Editors: Wojciech Kolanowski and Malgorzata Ziarno

Received: 4 April 2025
Revised: 11 May 2025
Accepted: 19 May 2025
Published: 22 May 2025

Citation: Hanga-Farcas, A.; Fritea, L.; Filip, G.A.; Clichici, S.; Vicas, L.G.; Frum, A.; Muresan, M.E. Comparative Antioxidant and Anti-Inflammatory Activity of Ellagic Acid and *Juglans regia* L. in Collagenase-Induced Osteoarthritis in Rats. *Appl. Sci.* **2025**, *15*, 5814. https://doi.org/10.3390/app15115814

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1. Introduction

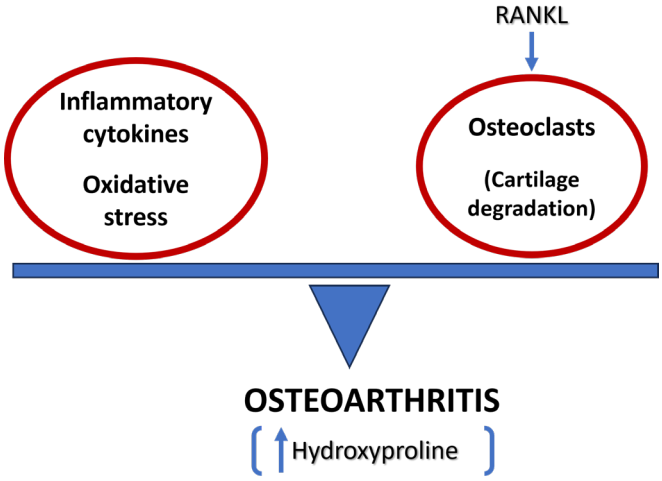
Osteoarthritis (OA) is a degenerative pathology of the joints, affecting the entire joint: cartilage, synovium, subchondral bone, meniscus, ligaments, joint capsule and soft tissues. The risk factors that lead to its occurrence are advanced age, joint trauma, obesity, and overuse. The main symptoms are pain and functional impotence, swelling and joint instability. In the advanced stages of the disease, the symptoms limit the activity of patients, with serious effects on the physical and mental health of the patient, as well as economic and social aspects [1].

Articular cartilage is a specialized connective tissue made up of chondrocytes that produce collagen, proteoglycans, and elastin fibers essential for maintaining the integrity of the joint [2]. Articular cartilage is a connective tissue that lines the bone at the joints, with the function of lubricating the smooth surface and facilitating movement. Articular cartilage has a limited capacity for healing because it lacks nerves and blood vessels; therefore, maintaining intact articular cartilage is important in preventing OA [3].

One specific characteristic of osteoarthritis consists of collagen synthesis/collagen degradation imbalance. The production of proinflammatory and fibrotic mediators which are involved in an inflammation process is generated by the reaction of macrophages and fibroblast-like synoviocytes with molecules in damaged hyaline cartilage [4]. Synovial inflammation will increase the production of proinflammatory cytokines, such as IL-6, tumor necrosis factor (TNF) and prostaglandins that contribute to cartilage degradation and exacerbate joint damage [5]. IL-6, a proinflammatory mediator, is actively involved in the development of OA. Elevated serum or synovial fluid levels in patients with OA are associated with increased incidence and severity of the disease. IL-6 induces matrix-degrading enzymes, thereby significantly contributing to the cartilage pathology development. However, it is also involved in the anti-catabolic factors expression with a protective role [6].

Tumor necrosis factor-α (TNF-α) and IL-6 indirectly induce osteoclastogenesis through the RANK/RANKL signaling pathway. Increased RANKL in the cartilage matrix allows its binding to its receptor, RANK, on the surface of preosteoclasts, which will determine the production of mature osteoclasts responsible for resorbing subchondral bone and will implicitly lead to the appearance of osteochondritis [7]. RANK/RANKL signaling pathway presents a specific role in osteoclast differentiation and activation, and it is therefore considered an important factor in the occurrence and progression of OA. Elevated RANKL levels in the cartilage matrix trigger many molecular events, causing progressive cartilage damage. The receptor from the preosteoclast cells surface (RANK) is bind by its ligand (RANKL), causing cells differentiation and maturation [8]. Another important biomarker, hydroxyproline, is an amino acid that represents one of the main components of the collagen protein, ensuring the stability and shape of the cartilage [9].

The important influence of those biomarkers in the OA development is represented in Scheme 1.



Scheme 1. Pathogenesis mechanism in OA with the influence of oxidative stress, cytokines and RANKL.

Collagenase is an enzyme that acts on the peptide bonds in collagen, breaking them. Intra-articular injection of collagenase leads to the degradation of articular collagen, which causes the appearance of OA, which gradually sets in 6 weeks after the injection of two doses of 500 U of collagenase. Then, clear signs of OA appear, such as deep erosions of

the cartilage matrix with areas of exposure of the subchondral bone, cartilage cracks with hypertrophy and inflammation of the synovium [10].

Currently, the main treatment methods for OA focus on combating pain, and they are mainly represented by anti-inflammatory and anti-allergic medication which can present a series of adverse reactions; however, they focus on treating the symptoms and not the cause [11]. The common treatment options for OA include drugs such as non-steroidal anti-inflammatory drugs, steroids and opioids with their specific side effects. Therefore, in recent years, many research studies focused on natural pharmaceuticals and nutraceuticals which may be explored as alternative options, being effective, safe and cost-efficient, with great potential in the treatment of OA, especially due to their anti-inflammatory and analgesic effects, anti-oxidation, regulation of chondrocyte metabolism and proliferation, and cartilage protection [12–16].

Traditional Brazilian plants were reviewed by Bezerra et al., including 24 species with anti-arthritic and anti-osteoarthritic activities (investigated in preclinical models), and 14 species (in randomized clinical trials), their effects being ascribed to phenolic compounds (such as flavonoids) [17]. Plant-derived polysaccharides have shown great effects in anti-inflammation, anti-oxidation, the regulation of chondrocyte metabolism and proliferation and cartilage protection, demonstrating significant potential in OA treatment. Polysaccharides have been found to reduce the expressions of pro-inflammatory cytokines (such as NF-κB, IL-1β, IL-6, TNF-α, COX-2, iNOS) and to have antioxidant effects (by down-regulation of SOD, MDA, CAT activities, inhibition of caspase pathway) in studies performed on cells culture and animal models [13].

Achyranthes bidentate extract, a traditional Chinese medicinal plant with saponin components, presented protective effects against inflammation induced by IL-1β in human chondrocytes due to the reduction of proinflammatory factors expression (such as IL-6, TNF-α, COX-2, iNOS, PGE2, NO) and the inhibition of NF-kB activity [18]. Plants from *Arecaceae* family present potential as anti-OA agents due to their metabolites (galactomannan, fatty acids, flavonoids, phenolic acids, polyphenols and steroids) which show anti-inflammatory and chondroprotective effects [12].

In an in vivo study on rats, Al-Shammari et al. showed the anti-arthritic potential of walnut oil, which decreased anti-arthritic biomarkers and improved morphological appearance [19]. The effect of walnut extract included in the daily diet demonstrated beneficial effects on rheumatoid arthritis in rats, such as decreased cellular infiltration, bone erosion and paw inflammation (histological examination); and a negative regulation of the RANKL-OPG pathway (gene expression analysis) [20]. In another study, the same authors showed the antioxidant role of walnuts, without hepatic or renal side effects [21]. Another study validated the anti-inflammatory effects of walnut leaves extract in rheumatoid arthritis, both in acute and chronic inflammation. The extract possessed anti-inflammatory and immunomodulatory properties, by decreasing pro-inflammatory markers (TNF-α, IL-1β, IL-6, NF-κB, COX-2 and PGE2), but also by up-regulating anti-inflammatory ones (IL-4) [22].

EA may help modulate osteoclast activity, reducing bone reabsorption, while promoting osteoblast activity to support bone formation, potentially helping to prevent bone loss [23]. Ellagic acid, a natural polyphenol found in many plants products, with anti-inflammatory and antioxidant proprieties was also used in the in vitro and in vivo experiments illustrating anti-inflammatory effects by inducing OA protectiveness [18]. The in vitro study on human chondrocytes shown that IL-6 and TNF-α levels were significantly decreased in the group treated with EA in a dose-dependent way [18].

According to our previous study, where we performed the phytochemical composition analyses of walnut extract [24], we found the presence of ellagic acid, which motivated

us to carry out this comparative study between the effects of ellagic acid and walnut extract. The therapeutic effects of *Juglans regia* L. can be attributed to its bioactive constituents, including ellagic acid, quercetin and catechins. These compounds activate the Nrf2 signaling pathway, which plays an essential role in cellular defense mechanisms against oxidative stress and inflammation. Our results complement other studies that have reported that walnut extracts inhibited the production of reactive oxygen species (ROS) and modulate proinflammatory pathways, further supporting their potential in the management of osteoarthritis [25].

Therefore, this study aimed to comparatively quantify the effect of JR leaf extract and EA in rats with induced OA by assessing oxidative stress, pro-inflammatory cytokines and bone regeneration parameters, in comparison with indomethacin and placebo effects.

2. Materials and Methods

2.1. Reagents

Collagenase type II (Clostridium histolyticum, type II) was purchased from Sigma-Aldrich (St. Louis, MO, USA), 4 mg was dissolved in saline solution and filtered with a 0.22 mm membrane, and the obtained solution was used for intra-articular injection [26]. RANKL, IL-6, TNF-α and hydroxyproline ELISA tests were purchased from Elabsicence (Houston, TX, USA). Xanthine, xanthine oxidase and Bradford reagent were procured from Sigma-Aldrich (Sigma–Aldrich Chemicals GmbH Inc., Seelze, Germany), whereas 2-thiobarbituric acid and EDTA-Na_2 were obtained from Merck KGaA (Darmstadt, Germany); these reagents were used for the determination of malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT).

2.2. Juglans regia L. Extract—Preparation and Characterization

In our previous study [24], we already presented the details about preparation and phytochemical characterization of JR extract: *Juglans regia* L. leaves, purchased from a local tea company (Fares, Orăștie, Hunedoara, Romania), were ground to a fine powder, then to 5.00 g of powder, 50 mL of 70% (*v*/*v*) alcohol was added and the mixture was kept at room temperature for 10 days. After extraction and filtration, the fluid extract was evaporated to dryness, with a yield of 86%. According to the HPLC-UV analysis, the extract composition was as follows: (+)-catechin (322.01 ± 1.49 µg/g d.w.), caffeic acid (878.15 ± 1.50 µg/g d.w.), cinnamic acid (0.50 ± 0.02 µg/g d.w.), ellagic acid (0.34 ± 0.02 µg/g d.w.), ferulic acid (218.55 ± 1.42 µg/g d.w.), gallic acid (23.02 ± 0.11 µg/g d.w.), quercetin (328.11 ± 1.39 µg/g d.w.), resveratrol (56.92 ± 1.64 µg/g d.w.), rutin (61.62 ± 0.53 µg/g d.w.) and syringic acid (52.88 ± 0.75 µg/g d.w.) [24]. Total polyphenol content (144.96 mg GAE/g d.w.), flavonoid content (102.74 mg QE/g d.w.) and the antioxidant capacity (IC50 of 30.69 µg/mL extract) were also assessed [24].

2.3. Experimental Design

The in vivo study was performed on 48 female Wistar rats, 3 months old, weighing 150 ± 10 g, divided into 2 groups, A (2-week treatment) and B (4-week treatment), each group containing 4 subgroups: the first subgroup was treated with JR extract, the second with EA, the third with indomethacin and the fourth with placebo (in the figures denoted as VEH = vehicle) (n = 6). The animals were obtained from the Biobase of the University of Medicine and Pharmacy, Cluj-Napoca. Under general anesthesia with 90 mg/kg b.w. ketamine and 10 mg/kg b.w. xylasine intraperitoneally administered, the animals were injected with 25 µL of collagenase type II (500 U/mL) parapatellar at the right knee with a 26G needle, on day 1 and day 4 in order to induce OA [10].

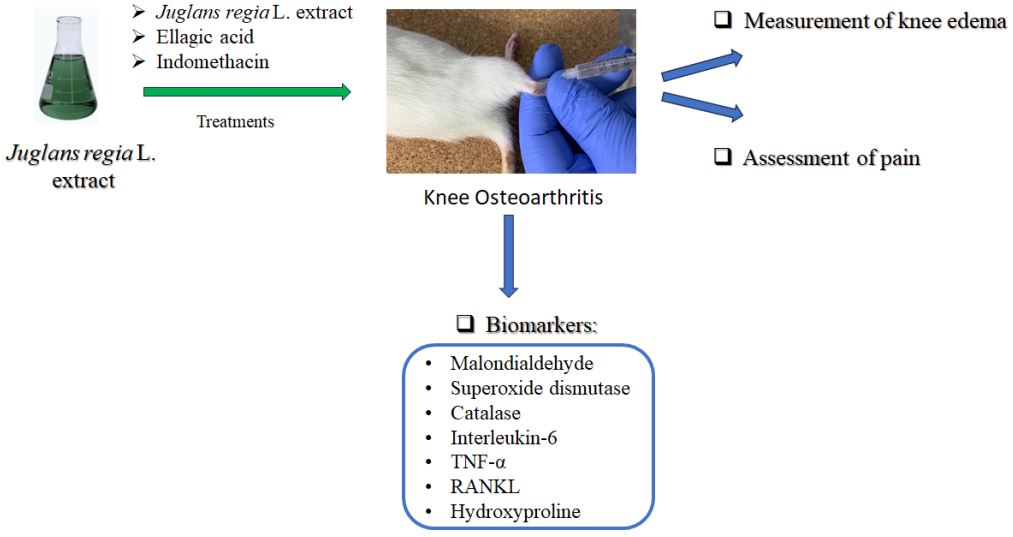
2.4. Measurement of Knee Edema and Assessment of Pain

The edema was measured above the knee by using water plethysmometer (UGO Basile 7140, Comerio-Varese, Italy) on both knees: right knee (where OA was induced) and left knee (used as control) on day 0 and 8 [27–29].

Randall–Selitto test was used for determining secondary mechanical hyperalgesia using an analgesimeter (UGO Basile 1247, Comerio-Varese, Italy). The right knee was placed on a flat surface while a blunt indicator was applied to the dorsal surface at a constantly increasing pressure, the pressure measured at that moment the animal withdraws its foot from the device was noted in grams, necessary to cause the withdrawal of the paw; as a control method, the test was also performed on the left knee. Measurements were performed on days 0 and 8 [30].

Starting on week 6, when histological changes of OA are most conclusive [31,32], the animals were administered treatments by gavage: groups 1A and 1B received JR extract 50 mg/kg, groups 2A and 2B received EA 50 mg/kg, groups 3A and 3B received indomethacin 2.5 mg/kg, and groups 4A and 4B received vehicle [33]. Three weeks before the experiments, the animals were acclimatized and kept in clean cages with access to food and water at a temperature of 20–24 °C, with a humidity of 40–60%, in a 12 h light and 12 h dark cycle. The same conditions were maintained throughout the experiment. The animals in group A were sacrificed after 2 weeks of treatment, and those in group B after 4 weeks of treatment, and blood samples were collected for biochemical investigations. We administered the treatment for 2 and 4 weeks after the induction of inflammation to simulate the changes in clinical practice in chronic forms of the disease. The experiments were performed according to the approved protocol of the Ethical Committee on Animal Welfare of the "Iuliu Hatieganu" University (16/9 November 2021) and of Cluj Sanitary Veterinary and Food Safety Directorate (290/9 February 2022).

The experimental design of this study is presented in Scheme 2.



Scheme 2. Graphical representation of the experimental design.

2.5. Biochemical Parameters

The redox status was evaluated by assessing MDA (as a marker of oxidative stress), and SOD and CAT (as markers of endogenous antioxidant defense), and the results re expressed as nmol/mL (for MDA) and U/g protein (for SOD and CAT activities). TNF-α, IL-6, RANKL and hydroxyproline levels were determined using the enzyme-linked immunosorbent assay (ELISA) technique, and the results were expressed in pg/mL.

2.6. Data Analysis

The statistic results for biochemical markers were analyzed using GraphPad Prism Software (version 10.4.2., San Diego, CA, USA), employing one-way ANOVA with Tukey's post hoc test, and with threshold significance level $p < 0.05$ (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3. Results

3.1. Measurement of Knee Edema and Assessment of Pain

The right leg volume measured 8 days after the first dose of collagenase was significantly higher 2.55 ± 0.02 compared to 2.00 ± 0.02 on day 0 before collagenase administration, demonstrating the development of inflammation at the knee level (Table 1). The edema increased at the right knee on day 8 by 27.43% in comparison with day 0.

Table 1. Plethysmometry and pain measurements.

	Day 0		Day 8	
	Left	**Right**	**Left**	**Right**
Plethysmometry measurements	1.99 ± 0.02	2.00 ± 0.02	2.06 ± 0.02	2.55 ± 0.07
Pain measurements	22.75 ± 0.31	23.42 ± 0.26	18.23 ± 0.67	7.42 ± 0.70

According to the Randall–Selitto test, a decrease in pain resistance occurs at 8 days after collagenase administration in right knee, from 23.42 ± 0.26 g to 7.42 ± 0.70 g on day 0, indicating a decrease in pain resistance due to edema in the right knee (Table 1). The right knee in day 8 was three times more sensitive to pain in comparison with day 0.

3.2. Biochemical Assays

Here, we evaluated the therapeutic effects of JR extract and EA on various oxidative stress biomarkers (MDA, SOD, CAT) and inflammatory markers (IL-6, TNF-α). Bone-related parameters (RANKL, hydroxyproline) in blood were also assessed at 2 and 4 weeks after oral administration of treatments.

Concerning the concentrations of MDA in all treated groups, both at 2 and 4 weeks of treatments' administration, no changes were noticed compared to the control group ($p > 0.01$) (Figure 1).

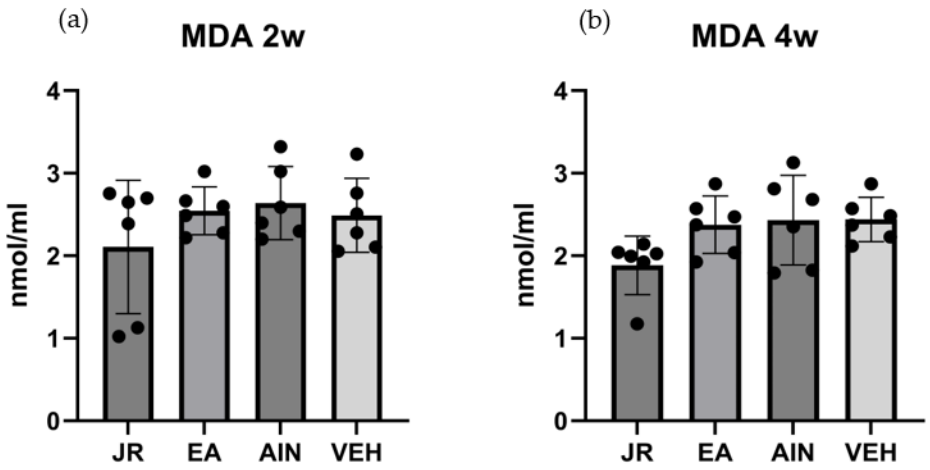


Figure 1. *Cont.*

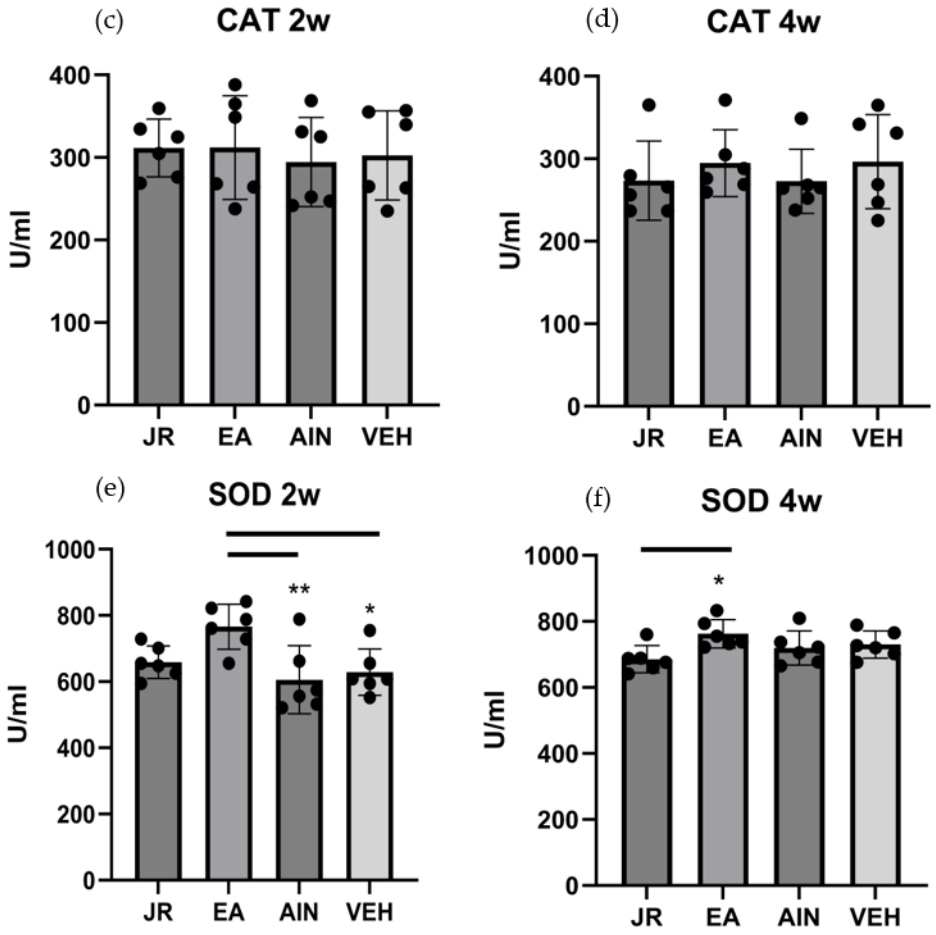


Figure 1. Malondialdehyde levels (**a**,**b**), CAT (**c**,**d**) and SOD (**e**,**f**) activities in blood at 2 weeks and 4 weeks of treatments' administration with EA, JR, AIN and vehicle in animals. Data are expressed as means ± standard deviation. The statistical significance of the difference between treated and control groups was evaluated with one-way ANOVA, followed by Tukey posttest, $p > 0.05$ (for MDA levels and CAT activity) and * $p < 0.05$ vs. JR or control group; ** $p < 0.01$ vs. AIN group (for SOD activity).

The CAT enzymatic activity was the same in all treated groups, at 2 weeks and at 4 weeks of treatments' administration, compared with the control group, ($p > 0.01$) (Figure 1). The activity of SOD was significantly increased in the EA-treated group compared with the indomethacin group ($p < 0.01$) and control group ($p < 0.05$) at 2 weeks and at 4 weeks; a slight increase in its activity compared with JR treatment group ($p < 0.05$) was registered (Figure 1).

IL-6 secretion decreased in the EA-treated group at 2 weeks of treatment in comparison with the control group, whereas there were no significant differences between the groups at 4 weeks (Figure 2).

TNF-α levels were reduced without statistical significance in the JR group only at 2 weeks of treatment, and there were no significant differences between the groups at 4 weeks (Figure 2).

In blood, RANKL levels decreased at 2 weeks of treatment administration in the EA-treated group compared to the indomethacin-treated group ($p < 0.01$). JR extract applied after induced inflammation slightly reduced the level of RANKL in JR group compared to the group which received indomethacin ($p < 0.05$). A significant decrease was maintained at 4 weeks in the JR and EA-treated groups in comparison to the experimental group with anti-inflammatory drug ($p < 0.001$). We registered a low level of RANKL in the group treated with JR compared to the animals treated with EA ($p < 0\ 05$) (Figure 3).

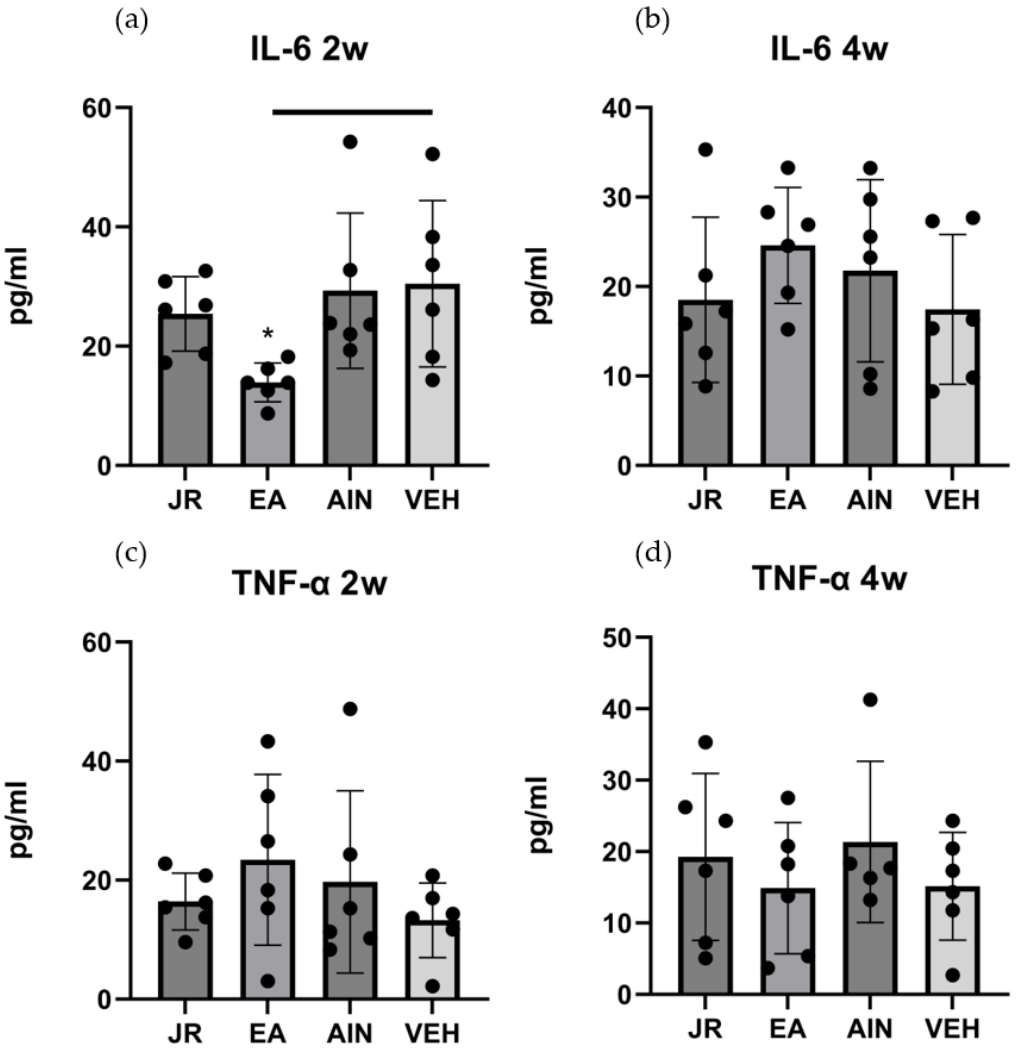


Figure 2. IL-6 (**a**,**b**) and TNF-*α* (**c**,**d**) in serum at 2 weeks and 4 weeks of treatments' administration with EA, JR, AIN and vehicle in animals. The statistical significance of the difference between treated and control groups was evaluated with one-way ANOVA, followed by Tukey posttest, * $p < 0.05$ vs. control group for IL-6 and $p > 0.05$ for TNF-*α*.

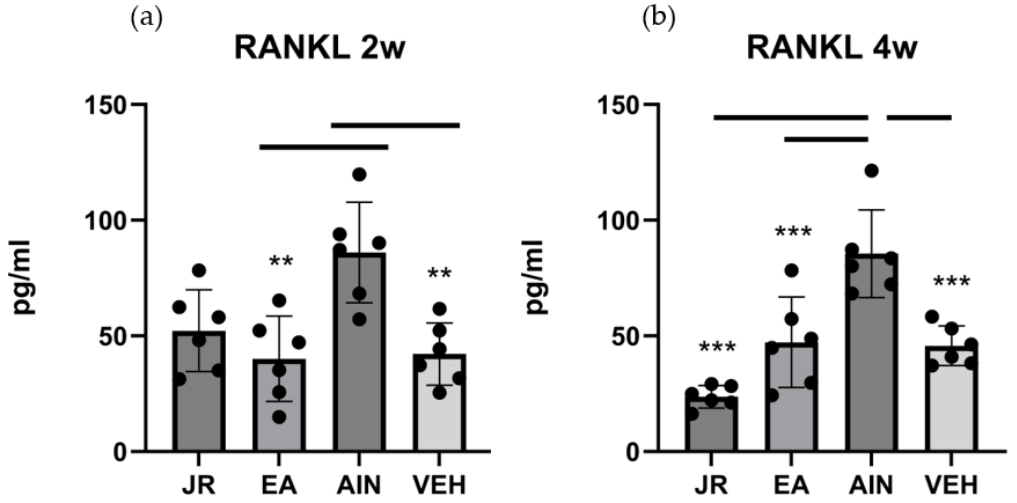


Figure 3. *Cont.*

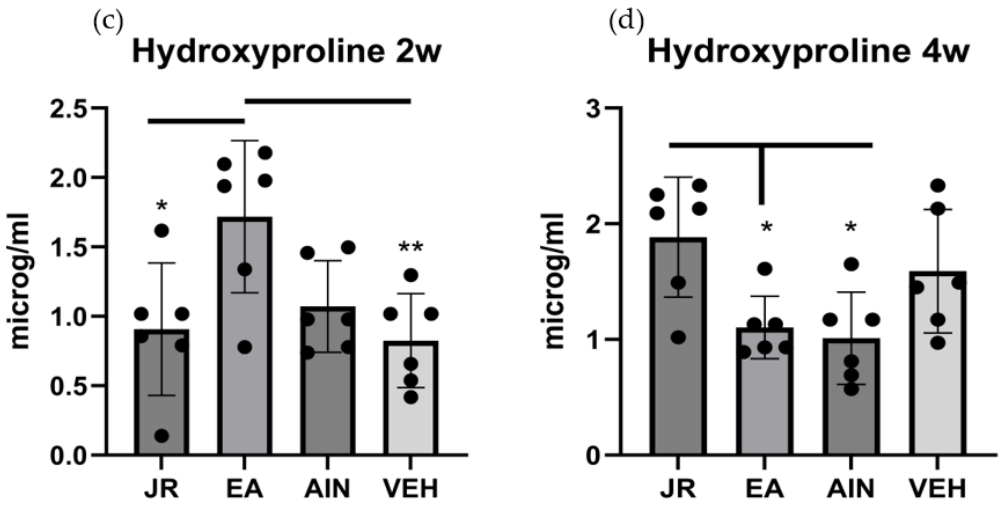


Figure 3. RANKL (**a,b**) and hydroxyproline (**c,d**) levels in serum at 2 weeks and 4 weeks of treatments' administration with EA, JR, AIN and vehicle in animals. The statistical significance of the difference between treated and control groups was evaluated with one-way ANOVA, followed by Tukey posttest, * $p < 0.05$ vs. AIN or EA groups; ** $p < 0.01$ vs. AIN or vehicle groups; *** $p < 0.001$ vs. AIN group.

Hydroxyproline levels were lower in the JR-treated group compared with the EA group ($p < 0.05$) at 2 weeks; meanwhile, at 4 weeks, the EA group recorded decreased concentrations of hydroxyproline compared to the JR group ($p < 0.05$) (Figure 3).

4. Discussion

Osteoarthritis is the most common joint disorder in humans, affecting millions of people globally and developing treatments to relieve pain and inflammation has been challenging. Long-term administration of pharmacological treatments such as non-steroidal anti-inflammatory drugs and/or corticosteroids has gastrointestinal, hepatic, blood, cardiovascular or renal adverse effects [34–36]. A wide range of plants were proven to present protection against OA and prevention of OA occurrence in in vivo and in vitro experiments, due to their phytochemical compounds.. Medicinal plants and their secondary metabolites were reported to present a positive effect in treating knee OA by various mechanism such as antioxidant activity, regulation of inflammatory stimuli, regulation of chondrocytes apoptosis and regulation of autophagy [37].

4.1. Antioxidant and Anti-Inflammatory Biomarkers

In our study, we performed the collagenase-induced osteoarthritic experimental model in rats to evaluate the anti-inflammatory and antioxidant effect of JR extract compared to EA as a compound with beneficial results demonstrated in OA.

The oxidative stress was evaluated by MDA level and by the enzymatic activity of CAT and SOD in blood. MDA is a well-known marker for the assessment of redox imbalance which leads to an increase level of the reactive oxygen species (ROS). Catalase is an antioxidant enzyme responsible for the conversion of hydrogen peroxide in water and oxygen, while SOD is an important enzymatic scavenger of oxygen radicals. The results of our work showed that there were no changes in MDA concentrations across all treated groups in comparison with the control. After 2 weeks of EA administration, SOD activity had significantly increased. Additionally, a favorable effect of antioxidant defense was also shown in SOD activity in JR-treated animals with OA.

Inflammatory cytokines, such as TNF-α, IL-1β and IL-6, besides exhibiting their pro-inflammatory activity, also proved to promote osteoclast activity leading to bone loss and

resorption. TNF-α also suppressed osteoblast function, while IL-6 boosted RANKL synthesis by osteoblasts [38]. Concerning the effects on pro-inflammatory cytokines, JR and EA diminished the secretion of TNF-α and IL-6 compared to the control group at 2 weeks of treatments.

The effect of *Bixa orellana* ethyl acetate fraction (BoEA) and ellagic acid was evaluated by Santiago et al. on rats with monosodium iodoacetate-induced OA, leading to a reduction in inflammation. The levels of the proinflammatory cytokine TNF-α were suppressed in the groups treated with BoEA and EA, similarly to the indomethacin-treated group. The levels of the anti-inflammatory cytokine IL-10 was increased in the groups treated with BoEA, EA and indomethacin, illustrating better inflammation control in those groups [39].

Papoutsi et al. demonstrated the anti-inflammatory effect of JR methanolic extract and of EA on endothelial cells by decreasing the TNF-a concentration, as well as the mineralization effect on osteoblasts by inducing nodule formation [40]. In comparison, in our study, TNF-α levels were decreased in the JR and EA groups only at 2 weeks of treatment. Meanwhile, in our previous work, the mineralization effect was observed in EA and JR groups due to accumulation of calcium and phosphorus, respectively [24].

Guava leaf extract rich in EA administered to rats with OA through diet suppressed the development of OA [41]. EA treatment improved arthritis-associated pathology in an experiment on rats with induced-arthritis, serum TNF-α levels decreased in the EA-treated group than in the control [42]. In a clinical study conducted on women with knee OA, treatment with pomegranate peel extract rich in EA, demonstrated an anti-inflammatory effect by reducing serum levels of inflammatory markers [43].

Epimedin C, a natural flavonoid compound found in Epimedium genus, markedly reduced the levels of TNF-α, IL-6, NO and PGE2 and effectively attenuated iNOS and COX-2. The antioxidant activity was also recorded by notable reduction in ROS production; meanwhile, MDA was significantly decreased and SOD expression was increased [44]. The same results concerning the suppression of inflammatory factors and the inhibition of ROS production were obtained by using pretreatment with *Oldenlandia diffusa* extracts [45].

An intestinal metabolite of EA, urolithin B, presented anti-inflammatory effects and cartilage-protective properties in a study performed on human chondrocytes by inhibition of inflammation produced by IL-1β and TNF-α [46]. While evaluating the effects of EA on IL-1β-induced oxidative stress in chondrocytes, a decrease in MDA and SOD was observed in the EA groups in comparison with the control groups [47]. In a controlled clinical trial, where patients with knee OA were treated with turmeric extract or paracetamol, it was noticed that C-reactive protein and TNF-α concentrations in blood were lower in the turmeric extract-treated group compared to the paracetamol group [48].

4.2. Osteogenic Biomarkers

Hydroxyproline is the main amino acid component from collagen, and its determination is an important biomarker for bone resorption and cartilage degradation. RANKL binds to its RANK receptor on osteoclast precursors, thus promoting osteolysis in subchondral bone at the onset of OA. In our study, EA decreased hydroxyproline at 4 weeks and decreased RANKL at 2 and 4 weeks, showing reduced osteoclastogenesis and decreased hydroxyproline content, confirming the beneficial effect on OA. The extract of JR decreased RANKL at 4 weeks, being the lowest concentration and maintained a low level of hydroxyproline at 2 weeks.

Elbeialy et al. demonstrated in a study that hydroxyproline serum level increased only in OA and not in rheumatoid arthritis at an early stage [49]. In an in vivo study, animals treated with modified Bushen Huoxue decoction for knee OA reported decreased

serum and urinary levels of hydroxyproline and MDA, while the level of SOD in serum and synovial tissue was higher [50].

In a study, rats with arthritis were subsequently treated with triphala, an Indian compound rich in EA, obtained from three plants (*Terminalia chebula* Retz., *Terminalia bellerica* Roxb and *Emblica officinalis* L.). The results showed that triphala normalized urinary hydroxyproline levels and reduced TNF-α, IL-6 and RANKL levels in affected tissue [51].

As an overall summary of our study, the antioxidant biomarker SOD was significantly increased in the EA group at 2 weeks, while the pro-inflammatory cytokine IL-6 was diminished in the EA group and TNF-α in the JR group at 2 weeks. Concerning the osteogenic biomarkers, the results of the present study show that in the experimental group treated with EA, RANKL level decreased at 2 weeks of treatment. Its effect was persistent at 4 weeks, in comparison with animals treated with indomethacin, suggesting a beneficial anti-osteoclastogenesis action. The JR extract presented a reduced expression of RANKL.

According to the obtained data, our study is a preliminary one that tries to highlight the effect of ellagic acid and JR extract in experimentally induced osteoarthritis. In order to confirm its effectiveness, future studies involving multiple doses, longer treatment periods, and examination of more parameters are needed, so as to reflect the mechanisms involved.

5. Conclusions

Osteoarthritis, a degenerative pathology of the joints, is mainly found in the elderly, and due to the aging of the global population, its prevalence is expected to increase; therefore, its management is a quite well-studied topic. In our work, EA significantly enhanced SOD activity, reduced systemic inflammation and diminished RANKL levels, in parallel with raising the levels of hydroxyproline. In the JR-treated group, the extract significantly decreased RANKL levels in comparison with the indomethacin group, especially at 4 weeks, suggesting a positive effect on osteoclastogenesis. JR extract administered for 2 weeks maintained a low level of hydroxyproline, indicating the need for longer treatment with this compound in OA or using different doses. Although medicinal plants have become a promising adjunct to conventional therapies due to their wide range of pharmacologically active compounds and an increased interest in complementary alternative medicine, further studies are necessary in order to establish the proper dose and time treatment in inflammatory diseases for optimal results.

Author Contributions: A.H.-F.: conceptualization, investigation, writing—original draft preparation and data interpretation; L.F.: methodology, investigation, and writing—review and editing; G.A.F.: writing—review and editing, supervision, and data interpretation; S.C.: methodology, formal analysis, and data curation; L.G.V.: project administration, funding acquisition; supervision; A.F.: methodology and investigation; M.E.M.: supervision, review. All authors have read and agreed to the published version of the manuscript.

Funding: The APC was supported by the University of Oradea.

Institutional Review Board Statement: 290/09.02.2022 (Cluj Sanitary Veterinary and Food Safety Directorate), AVZ16/09.11.2021 (UMF Iuliu Hatieganu Cluj-Napoca).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to express their gratitude to Remus Moldovan and Nicoleta Decea from the Department of Physiology, University of Medicine and Pharmacy "Iuliu Haţieganu", Cluj Napoca, Romania, for their technical support.

Conflicts of Interest: The authors declare no conflicts of interest.

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